REMARKS

Currently claims 1-8, 10, 12, 14-22, and 63-69 are pending in the present application. Claim 69 has been amended. Although applicants consider claim 69 to be clear and definite, to expedite prosecution, applicants have amended claim 69 to depend from claim 3. Applicants request entry of these amendments and response. If the Examiner does not allow the claims and issues an advisory action, applicants intend to file an appeal and as such require that these claims be entered.

Claim Rejections – 35 U.S.C. § 112

The examiner has rejected claim 69 under 35 U.S.C. § 112, second paragraph as being indefinite by its claim term: "at least 25 times longer than conventional non-liposomal formulations when tested in Swiss albino mice at equivalent doses." Claim 69 has been amended to now depend from claim 3, which depends from claim 1, which indicates that it is a process for manufacturing long circulating liposomes. Claim more narrowly defines the term "long circulating" and it uses language found in the specification. In addition, Examples provided in the specification provide guidance on the testing of liposomes manufactured by the present invention containing a therapeutic or diagnostic agent against conventional non-liposomal formulations of that same therapeutic agent (in this case Doxorubicin). These examples are standard tests routinely run by those skilled in the art for testing liposome stability (i.e. circulation times). See Dr. Daftary Declaration at para. 11-17.

The Examiner seems to take issue with the fact that comparisons were made with a liposome containing doxorubicin against a non-encapsulated form of doxorubicin (ADRIAMYCIN), whereas the claim does not recite any active ingredient. Claim 69 now depends from claim 3, which recites that the process of manufacture further comprises loading the liposomes with a therapeutic or diagnostic agent. This should alleviate the Examiner's concerns as the claim now recites the presence of an active ingredient.

The Examiner also seems to take issue with the fact that the claim compares a liposome containing an active ingredient against the active ingredient per se (in other words in a non-liposomal formulation) instead of making a comparison against another liposomal formulation. A patentee can make any comparison he desires and claim it, as long as it is clear, understandable and is definite (and thus meets the 35 U.S.C. §112 requirements). Just because

the Examiner wants to see a different comparison is not a basis for issuing a 35 U.S.C. §112 rejection. Further, as argued before and explained in the attached Declaration, the standard of comparison used by the patentee is widely used and accepted in the field. The Examiner has cited Slater in the office action and it is informative to note that Slater even compares liposome entrapped MPE Campotheticin with the drug administered in the free form. As Dr. Daftary indicates in his Declaration, it is common and expected in the industry to test liposomes by measuring their ability to maintain their contents while in circulation. Thus, one has to put some compound into the liposome to test the liposome's ability to keep its contents within the liposome shell. As Dr. Daftary indicates, one simply does not test empty liposomes for their stability. In addition one does not test a liposome against a "standard liposome" because there is no such thing as a "standard liposome" in the industry. See Declaration at para. 16-19. Dr. Daftary indicates that there is no standardized liposome because liposome properties vary according to their contents and their method of preparation. Liposomes and their behavior (stability while in circulation in the body, i.e. length of circulation time; ability to carry certain active ingredients without leaking, storage length, ability to entrap certain therapeutics efficiently) all depend on multiple factors, and all of these factors can work together, or against each other, or in some cases work together synergistically. Some of these factors include: the liposome components (i.e. the phospholipids, lipids, sterol, PEG, etc. and their amounts and ratios used); the solutions used to make (like hydration medium used for making liposomes from phospholipids; buffers for dialysis in removing ammonium sulphate from extraliposomal hydration medium) and subsequent solutions used for loading active matter into the liposomes; the conditions when making or loading the liposome; the conditions while loading an active ingredient for testing the liposomes; and the amount of the solutions used; etc.

Dr. Daftary also indicates that the art of liposome manufacture is unpredictable because so many factors can be chosen and used in the manufacture and their interplay produces unpredictable results. Hence, the industry does not recognize a "standard" liposome. Dr. Daftary provides an example: if two groups made a liposome composed of the same phospholipids and same sterols, the characteristics of these liposomes most likely will differ if they are made by a different process and especially if they were made with different solutions and different amounts of solutions. See Declaration at para. 19.

Accordingly, applicants submit that the Examiner has failed to consider the industry and the industry's usual methods of testing and studying liposomes. Applicants respectfully submit that one skilled in the art would find the claim clear and understandable, and it clearly indicates with particularity the invention. See Declaration at para. 20.

Dr. Daftary also points out that even the references cited in the Office Action show that there is not a standard liposome and further that what may be perceived by the Examiner as minor changes, dramatically effect the properties of the liposome. See Declaration at para. 19.

For example, Hong demonstrates that there is not a "standard" pegylated liposome. In Hong, the pharmaceutical properties of pegylated or non-pegylated liposomes vary from preparation to preparation. Amongst the pegylated liposomes, the extent of pegylation, the molecular weight of PEG used, and the manner of pegylation make a difference. Thus, Hong shows that changing the amount of one component changes the property of the liposome. See Declaration at para. 26.

As another example, Forssen also shows that changing one small component in the manufacture of liposomes can alter their behavior dramatically. Forssen notes that changing from one ammonium salt to another ammonium salt (e.g. from ammonium tartrate to ammonium sulphate) caused the entrapment procedure to drop in efficiency by over 50%. See Declaration at para. 27.

Thus, contrary to the Examiner's belief, one can not simply interchange solutions, process steps, and liposome components and have any sort of predictable outcome. See Declaration at para. 27.

The question is why is this property (long circulation) of the liposome claimed -- because this property of the liposome defines its structural difference from other liposomes of similar compositions and/or size. Dr. Daftary indicates that it is his belief that it is the structure of the liposome (which was achieved by combining the claimed liposome components with the claimed solutions in the claimed process) that makes the resulting liposomes long circulating (i.e. hold the therapeutic or diagnostic agent entrapped in it for longer time, and slowly deliver the active agent throughout this extended time, allowing it to act at the appropriate site, and reducing side effect toxicity). In other words, this property defines the structure of the liposome. Longer circulation increases usefulness of the liposome of the invention. Prior to the present invention, others altered the structure by having polyethylene glycol (PEG) on the surface of the liposomes. Pegylated liposomes, because of the polymer coating on the bilayers of the liposome membranes,

have been shown to hold the therapeutic or diagnostic agent for a longer time than non-pegylated liposomes by avoiding RES uptake. In contrast to using PEG, the present invention has achieved long circulation by altering the liposome's structure by using the claimed method of manufacture combined with the specific claimed liposome components and solutions. See Declaration at para. 21.

The Examiner seems also to take issue with the fact that ADRIAMCIN is a solid. The Examiner's argument that ADRIAMYCIN is "a solid and one can make different forms of non-liposomal compositions such as emulsions. . . . ." shows that the Examiner has ignored the teaching of the specification, which clearly shows how the ADRIAMCYIN was prepared for the testing. See para. 101 of the published specification.

Accordingly, applicants submit that claim 69 is not indefinite and that all of the 35 U.S.C. §112 requirements have been met, and request withdrawal of this ground of rejection.

CLAIM REJECTIONS – 35 U.S.C. § 103

Applicants note that the claimed process of manufacture recites four groups of elements all necessary for the desired result of a non-pegylated long circulating liposome: 1) the components of the liposomes (i.e. no polyethylene glycol (PEG), certain phospholipids (i.e. DSPC, HSPC, or mixtures thereof), and sterols); 2) the various process steps; 3) the use of certain solutions (i.e. hydration media of ammonium sulfate and sucrose; dialysis solution of sucrose-histidine); and 4) recited amounts of certain solutions in conjunction with the liposome components (10-35 ml of hydration media for each mole of phospholipid). All four of these (components, steps, solutions and amounts) act synergistically to produce long circulating non-pegylated liposomes. See Declaration at para. 22. The combination of these components, solutions, recited process steps and amounts of certain solutions, unexpectedly provided a long circulating liposome that does not require the presence of PEG to achieve the liposome stability. See Declaration at para. 21. In the case of liposome manufacture, it is well known that one can not simply take one step from one method and substitute with another step or solution from another method and achieve the same or results, especially when there are variations in the components and the solutions. See Declaration at para. 23-25. One of ordinary skill in the art would understand that you can not simply mix and match or substitute various components, steps and solutions and achieve any predictable result. See Declaration at para. 23-26. The role of the

quantity of hydration medium was explored along with exploration of all other elements as discussed above in the instant invention to result in a synergistic property of increasing circulation time of the liposome prepared by the entire process. Examples 2 and 13 of the present appliction clearly demonstrate that with other features being same, the reduction in the volume of the same hydration medium is effective in increasing circulation time. See Declaration para. 28.

Dr. Daftary has said in the Declaration that the same effect is not produced on liposomes by using the same quantity of hydration media when the composition of hydration media is different. The composition of hydration media and volume of hydration media used have varying effects not independent of each other. Similarly what is being hydrated is also part of the reason of deciding on hydration volume. If volume A is used for hydrating DSPC, the same volume may not be good for hydrating DSPC + Cholesterol, or EPC. A person of ordinary skill in the art, being aware of this fact, would not pick up some volume of hydration medium for a lipid composition mentioned in one reference and apply it for hydrating another lipid composition mentioned in another reference expecting to get liposomes having properties similar to the liposomes of the present invention. For example, the hydration volume of Janoff would not be considered as it is for EPC as phospholipid, and for sodium chloride, HEPES and sucrose constituting the hydration medium. Further, Janoff would not be considered by one ordinarily skilled in the art for combination with Slater, which has Hydrogenated Soya phosphatidylcholine + Cholesterol and PEG-DSPE as phospholipids for hydration, and ammonium sulphate and dextran sulphate-ammonium salt solution as hydration medium. Similarly, the volume of hydration medium used by Clerc uses weak acid salts and is not suitable for the hydration medium used by Forssen, which uses a strong acid salt (ammonium sulphate) and which has no combination of ammonium sulphate and sucrose, as in claim 1 of the present invention. Hence the combinations suggested by the examiner to show that use of such volume of hydration medium in claim 1 is obvious, is not persuasive and disregards how one skilled in the art would review these references. See Declaration at para. 23.

Dr. Daftary's declaration shows in a tabulated format (Table I) that none of the references cited by examiner teach or suggest the use of ammonium sulphate-sucrose as a hydration medium and their amount per mMole of phospholipid as in claim 1, and hence any combinations based on these references would not make the present invention obvious. Any suggestion to take

ammonium sulphate from the hydration medium of one reference and sucrose from the hydration media of another reference to show that this makes the invention obvious is not persuasive. Similar is the case with volume of hydration medium used and a histidine–sucrose dialysis medium. In Table II, Dr. Daftary has summarized and pointed out why the reasoning for the three combinations of the references that examiner has cited to make the instant invention obvious are not persuasive. Tables I and II are also attached at the end of this response to the Office Action. The Examiner has attempted to find each and every limitation of the claims by mixing and matching portions of various references thoroughly disregarding how these elements were used in the cited art. The Examiner has attempted to argue that he has found all of the limitations. First, the Examiner has failed to actually find all of the limitations even with this mixing and matching and disregard for the actual or perceived feasibility to one ordinarily skilled in the art in these substitutions. This is evident by the fact that the examiner has had to resort to finding teachings "implicit" in the reference. Second, in his attempt to find all of the elements, the Examiner has ignored the fact that one skilled in the art would not have carried out this mix and matching analysis as it disregards the common knowledge and understanding in the field of liposome preparation.

The applicants will show below that each and every element has not been taught by the references, alone or in combination with each other. The Examiner has argued that applicants cannot show non-obviousness by attacking references individually where the rejections are based on combinations of references. Applicants respectfully point out that although applicants point out the deficiencies in each reference, applicants note that even taking all of the references together, no combination teaches each and every element. The attached chart (Appendix 1) , shows the deficiencies in each reference and it clearly reveals that even with the combinations proffered by the Examiner, there is a lack of teaching, suggestion or motivation to say that each and every element of the instant invention is obvious from such combinations. Further, applicants have argued and provided a Declaration of Dr. Daftary that shows one skilled in the art would not have even been motivated to combine these references, notwithstanding the combination still fails to show each and every element of the claims, as none of the references have any objective to make a liposome long circulating without pegylation.

I. Claims 1-8, 10, 12, 14-22 and 63-69 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Hong (Clinical Cancer Research, 1999), Janoff (US 4,880,635) and Papahadjopoulos (US 4,235,871), optionally in further combination with Barenholz (US 5,316,771).

The Examiner combines Hong, Janoff and Papahadjopoulos (and optionally with Barenholz) and alleges that they render the claimed invention obvious. To do so, they must together teach or suggest each and every claim element. These references do not teach or suggest each and every claim element, even when combined. Further, one skilled in the art would not be motivated to combine these references to arrive at the present invention. The deficiencies of the references and the lack of motivation to combine these references are discussed in more detail below.

A. Hong does not teach each and every element of the claimed invention.

Hong is deficient in its asserted teachings and uncured by the remaining references for at least the following reasons. As discussed in previous responses, Hong does not teach or suggest at least three elements of the claims: 1) an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose; 2) the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present (wherein the phospholipid is DSPC or HSPC or mixtures thereof, and is not pegylated as required by the claims); and 3) removing extra liposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

First, Hong focuses on the study of pegylated liposomes containing doxorubicin and whether the presence of polyethylene glycol (PEG) offers any advantage in the treatment of tumors. Hong does not teach, suggest or provide any motivation to design a novel method of liposome manufacture to produce a long circulating non-pegylated liposome. Mainly, Hong notes that there are differences between pegylated and nonpegylated liposome and even though PEGylation decreased the V_{ss} (steady-state volume of distribution) and clearance and increased the AUC (area under the concentration-time curve)(see Hong, page 3650, first column), the superiority in therapeutic effect of liposomal doxorubicin to free drug was not well explained merely by the higher AUC achieved. Hong goes onto to say that increasing the plasma AUC by PEGylation is not satisfactory. See id, page 3651, first column. There simply is no motivation

to make a long circulating liposome by using a different hydration buffer, by using a reduced amount of hydration buffer and by using a different dialysis solution (as required by the claim). At most, Hong suggests further study about different delivery routes to obtain increase local drug concentrations by local hyperthermia or by the use of immunoliposomes. See id.

The Examiner has remarked that: "The instant claim is not for tumor treatment." This remark is not relevant because, the liposomes of the present invention are eventually useful for tumor treatment and results obtained with the liposomes created by the method of claim 1, are shown in the specification. See also claim 42 of the instant invention as filed. It is only because of election/restrictions of groups of claims required by the Office Action such claims are outside this present application under examination. The product of the instant invention, based on the liposomes of claim 1 is the only one commercially available at this time.

In addition, Hong studies the effect of PEG and noted that "PEG altered the pharmacokinetic property of the DSPC/cholesterol liposomal doxorubicin." See Hong, page 3650, first column. Notably, Hong mentions other liposome systems (i.e. egg phosphatidylcholine/cholesterol liposomes) and notes that they act differently. This shows that one skilled in the art understands that liposomes made of different components behave differently and their interactions are not interchangeable with other liposomes, and nor are there properties predictable. See also Declaration at para. 16 and para. 19-25.

Second, in Hong, the contents were hydrated at 55 °C in an ammonium sulphate solution (250 mM at pH 5.0) and extruded through polycarbonate membrane filters of 0.1 to 0.05 μm pore size. Thus, Hong is using an ammonium sulphate solution for hydration whereas the present claimed invention uses a hydration medium consisting essentially of ammonium sulfate and sucrose. As such Hong does not teach this element of the claim.

Third, Hong does not teach that the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present. The Examiner has argued that "it would have been obvious . . . to determine as to how much hydration buffer is needed to complete the formation of liposomes." See page 4 of office action dated March 9, 2011. Determining how much buffer to complete the formation of liposomes is not the same as determining how much buffer is necessary to form long circulating non-pegylated liposomes. See Declaration at para. 28. In determining how much buffer is needed to form liposomes, one

normally uses an excess of liquid as one wants to have enough liquid to make sure the liposomes can form. There is no motivation to restrict the amount of liquid used. See id.

Fourth, Hong does not teach removing extra liposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

All of these deficiencies along with the Examiner's arguments and secondary references brought in to allegedly cure these deficiencies are discussed in more detail below.

Further, if we consider the combinations suggested by the Examiner which says that it is obvious to change the volume of hydration media in Hong to that used by Papahadjopoulos, we see that such a suggestion is not persuasive because:

a) The process of hydration is different from that in Hong, and it is not compatible with that in Hong. Papahadjopoulo's process is a two stage process and it goes through a gel stage. Further, the hydration in the presence of a solvent is not compatible with process of Hong (See Hong example 2).

b) The volume of the hydration media is outside of that in instant claim 1, which is for mMole of phospholipid and not per total lipid. This volume in both examples 1 and 2 is not just 1.5 ml as mentioned by the Examiner. The Examiner overlooks the fact that in Example 1 and 2, the rotary evaporation removes only the solvent (ether). Example 1 and 2 does not indicate formation of a dry lipid film but specifies evaporation up to the gel stage, which clearly is an indication that the aqueous mixture that is added is retained in the gel form (and not evaporated entirely). To this gel, again is added 1.5 ml of the buffer solution to form liposomes.

c) The hydration medium and phospholipids used in the two processes are different from instant claim 1. (Lipids contain phosphatidylglycerol).

d) The objective of Papahadjopoulos is to increase circulation time using a lipid polymer conjugate.

Similarly the Examiner combined Hong with Papahadjopoulos to show inclusion of histidine in the hydration buffer. This is not relevant as histidine is not used in the hydration buffer in the instant invention.

The examiner combined Hong with Janoff to argue that use of sucrose in the hydration media is obvious because Janoff is showing sucrose inside and outside liposome retains adriamycin during dehydration and rehydration of the liposome. The Examiner also used Janoff to argue that the amount of hydration buffer cited in the claims would be obvious. Dr. Daftary in his Declaration covered all these reasoning and summarized them in TABLE II of his declaration and has shown why the reasons given by the Examiner for such combinations are not persuasive.

The Examiner combined Hong with Barenholz for removal of ammonium sulfate from extraliposomal hydration media. This is not relevant as Barenholz does not use sucrose.

"an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose"

The Examiner has failed to show a reference teaching the use of a hydration medium consisting essentially of ammonium sulfate and sucrose, and to try and overcome Hong's deficiency the Examiner cited Janoff for teaching the presence of sugars to retain an active ingredient during dehydration and rehydration of liposomes.

First, applicants note that Janoff does not teach "an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose." Nor does Janoff even relate to a method of liposome manufacture that results in a non-pegylated long circulating liposome.

Janoff discloses the preparation of dehydrated liposomes by drying liposome preparations under reduced pressure in the presence of one or more protective sugars. Exemplified sugars include sucrose. The dehydration is conducted under vacuum with or without prior freezing of the liposome preparation. There is no reference to any ammonium salt or to any sulfate, let alone the use of an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose, as required by the claims. Reference is made to loading rehydrated liposomes using a concentration gradient created after rehydration. In the exemplified processes, 80 µmoles EPC was hydrated with 2 ml aqueous solution containing 150 mM sodium chloride, 20 mM HEPES and the respective sugar (see Janoff, column 8, lines 40-63).

The fact that sucrose or another sugar inhibits leakage of an encapsulated active material from dehydrated liposomes is no indication that it will prevent leakage from the liposomes prior

to dehydration or from the rehydrated liposomes after loading with the required drug or other active material. Further, there is no evidence to say that leakage of liposomes has any relevance to making a long-circulating of liposome. The purpose of the liposomes of the present invention is not to make the liposome survive the dehydration process without losing internal contents, it is to make a liposome that will slowly deliver the drug entrapped in it, and at the same time remain stable over a longer period in the circulating blood, and Janoff does not suggest such function of his liposomes as pointed out by Dr. Daftary. The instant invention uses the aqueous hydration medium consisting essentially of ammonium sulfate and sucrose to hydrate phospholipids to form liposomes, whereas Janoff uses 150mM sodium chloride solution containing 20mM HEPES and Trehalose (sucrose not mentioned specifically) to hydrate dehydrated liposomes. Hydration to form lipids versus hydration of dehydrated liposomes are quite different things and the processes are not interchangeable. See Declaration at para. 28.

There is no need for dehydration or rehydration of liposomes nor any recitation of such a step in Hong, or the present invention. Accordingly, one reading Janoff would not be motivated to use sugar along with ammonium sulfate in a phospholipid hydration medium, especially when the process does not involve dehydration. Janoff does not suggest that sugar addition will increase the circulation time but conversely, Janoff's abstract says it is for stabilizing liposomes during hydration and rehydration. There thus would be no motivation to combine Janoff with Hong. See Declaration at para. 29-31.

Despite all of the arguments offered by the Examiner, the real issue is whether Janoff suggests the use of sucrose with ammonium sulphate for hydration of phospholipid to form a liposome? No, he does not, and there is no suggestion to do so either. In Col. 8, lines 40 –43 where Janoff describes how vesicles ETV are prepared, it is clear that Janoff does not use sugar with ammonium sulphate during hydration of phospholipids for making liposomes, as required by the instant claims. Rather Janoff is using sodium chloride, and HEPES buffer with sugar (Trehalose specifically mentioned) for protecting liposome structure during dehydration. Further, there is no reasoning or motivation to pick only sucrose from a laundry list of sugars proffered by Janoff and combine it with ammonium sulfate to achieve a long lasting non-pegylated liposome when Janoff uses sodium chloride and HEPES buffer along with Trehalose (a sugar) for a different purpose altogether. Dr. Daftary in his declaration has given the insight as to the unique function of sucrose from other sugars. He says that the purpose of the liposomes

of the instant invention is not to make the liposomes impervious to leakage on dehydration, but rather it is to make a liposome that will slowly deliver the drug entrapped in it, and at the same time remain stable in the circulating blood. In other words, the liposome structure is designed to deliver the drug in this way by using sucrose in the hydration medium for the specific phospholipids, which may not happen with any other sugar and any other phospholipid. For Janoff any sugar is equally good, his requirement of the liposome is to withstand the dehydration/hydration process, he is not concerned with its stability in vivo as explained in the Declaration. Janoff tests stability of dehydrated liposomes (moisture less than 2%) stored in refrigerator up to 7 days. There is no indication whether it will stand in aqueous conditions while circulating in blood at body temperature. (See page 24). To reach the Examiner's conclusion, one would have to speculate in hindsight reasoning. It is not obvious to a person of ordinary skill in the art. See Declaration at para. 29 – 32.

The examiner also brings in Barenholz in an attempt to overcome the deficiencies of Hong. Barenholz also does not teach "an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose." Nor does Barenholz provide any motivation to practice any other process steps of the claimed invention. Barenholz relates to a procedure for loading amphiphatic drugs and chemicals into liposomes and does not relate to a process of manufacture of liposomes. Although Barenholz uses an ammonium gradient for loading the liposomes, he does not teach or suggest using a hydration medium of ammonium sulfate and sucrose (as required by the claims). Further, Barenholz does not teach using this hydration medium at a range of 10 to 35 ml for each mmole of phospholipid present to make a long circulating nonpegylated liposome. Merely using an ammonium gradient for liposome loading does not suggest, teach or motivate one to use a hydration buffer consisting of ammonium sulfate and sucrose to make a liposome. See Declaration, at para. 33. Liposome loading and the process of forming liposomes are two entirely different processes. Further, they are not interchangeable and nor are the results of changing different solutions in different steps predictable. One skilled in the art would not look to processes and solutions used to form or make a liposome and believe that they would be interchangeable and work with processes and solutions used to load liposomes once they are formed. See Declaration at para. 34.

"the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present"

To try and overcome Hong's deficiency relating to the amount of hydration media used the Examiner brings in Papahadjopoulos and argues that it teaches: "the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present" as required by the claims. Applicants reassert that the Examiner has miscalculated the amounts.

Calculation of volume of hydration buffer per mM of phospholipid

Papahadjopoulos does not teach the claim element where the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present. In Papahadjopoulos, the amount of phospholipid used is 50 μM (cholesterol is 50 μM) and there is 5ml ether in organic phase. In addition, there is 1.5 ml aqueous phase buffer plus 1 ml (~ 10 mg) from the alkaline phosphatase solution. Although it is not precisely clear how much water of this aqueous phase remains in viscous gel, applicants assume that the maximum is 2.5 ml. In addition, another 1.5 ml of aqueous phase buffer is added to the gel when it changes to liposomes. At that stage the maximum water content would be 3 ml. Thus, 3 ml of aqueous phase for 50 μM of phospholipids converts to 3,000 ml per 50 mM, or 60 ml per mM, which is clearly outside of the range of the claimed invention. Even if one were to guess that there would be some loss of water during intermediate evaporation, even if assuming that the aqueous phase may be less than 3 ml (such as 2ml), there would be 2 ml of aqueous phase per 50 μM of phospholipid. This converts to 2000 ml per 50 mM; or 40 ml per mM which is certainly outside the range of the instant invention. See Declaration at para. 35.

The Examiner's calculations contain an additional error in what is taught in Papahadjopoulos as he has not calculated ml of aqueous medium per millimole of phospholipid (as stated in the claims). Instead, the Examiner has calculated ml of aqueous medium for total lipids (which includes Cholesterol) and not just phospholipids as required by the claims. Therefore his calculations are erroneous. The claim clearly recites that that the hydration buffer is used at a range of 10 to 35 ml for each mmole of phospholipid present. The claim does not recite that other lipids or cholesterol fall within this range. Thus, it is incorrect to take lipids and

cholesterol into account when the claim clearly recites phospholipid. See Declaration at para. 36.

The Examiner also argued that it would have been obvious to determine how much hydration buffer is needed to complete the formation of liposomes. Applicants note that determining how much buffer (hydration medium) to complete the formation of liposomes is not the same as determining how much buffer is necessary to form long circulating non pegylated liposomes. See Declaration at para. 35. Determining how much buffer to complete the formation of liposomes does not lead one to consider how little is necessary to form a long circulating non pegylated liposome. In addition, it is a combination of all of the claimed process steps, claimed solutions, and claimed liposome components that work synergistically with the amount and make-up of the hydration buffer to achieve the long circulating non-pegylated liposomes. See Declaration at para. 35-36. Further, as Dr. Daftary explains, there is always extra liposomal hydration medium remaining after the hydration to form the liposomes. Thus, there is always an excess of the minimum required for the hydration. The claim language of "the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present" defines the total quantity of the hydration media used (including this reminder/excess hydration medium). So the claim is not simply describing the situation where someone merely adds a huge excess of hydration buffer to allow hydration of all of the liposomes but rather, the claim has recited a certain finite amount necessary to achieve hydration but also small enough to provide a tightly packed long circulating non pegylated liposome taking into consideration the other process steps, the make up of the solutions and the make up of the liposomes. Further, the claim also recites that ammonium sulphate is removed in a certain defined step with a certain defined solution: "removing ammonium sulfate from the extra liposomal hydration medium by dialysis using a sucrose-histidine buffer solution." See Declaration at para. 37.

The Examiner also brings in Janoff in an attempt to overcome Hong's deficiencies. The Examiner argues that Janoff teaches 80 micromoles of lipid with 2 ml of buffer (25 ml per mmole). Applicants note that Janoff is using egg phosphatidyl choline and not the claimed phospholipids (DSPC or HSPC) so Janoff does not teach the claimed element of "the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present" (emphasis added) as the present claims define phospholipid as either DSCPC, HSPC or

mixtures thereof. Further, Janoff is also not using the claimed hydration medium as Janoff is using sodium chloride, HEPES, and trehalose. Further, the conditions where Janoff's process is useful are not the requirements of the present invention. Janoff is useful for liposomes that are to be dehydrated, stored and rehydrated. Liposomes of the present invention have to be long circulating in the blood at body temperature and remain in aqueous environment releasing the drug slowly into the blood, which has nothing to do with freezing, dehydration, long term storage and rehydration as in Janoff. Therefore, there is no reason to expect any success on using sucrose from teachings of Janoff hydration medium and adding it to hydration medium of Hong.

Dr. Daftary notes that one of ordinary skill in the art would not be able to predict the interaction of sugar with the inside membrane of the liposome prepared by using egg lecithin, sodium chloride and HEPES buffer as Janoff teaches, and one could also not be able to predict that sucrose would interact with internal membrane of a pegylated liposome taught by Hong in the same way. Hence, there would be no motivation to combine Janoff with Hong. See Declaration at para. 39. One would not be able to predict the interaction of sodium chloride and HEPES buffer with sugars as used by Janoff to be the same as the interaction of sugars, especially when one process uses dehydration (Janoff) and the other (Hong) does not. See id.

One would not be motivated to combine Janoff's use of a solution comprising sugar, sodium chloride and HEPES with Hong's lipids of DSPC, Cholesterol and PEG, especially since Hong's liposomes were not dehydrated as in Janoff. Although both Hong and Janoff relate to liposomes, there would be no motivation to combine these references because Hong is focusing on comparing the effects of polyethylene glycol (PEG) on doxorubicin liposomes where Janoff is focusing on designing a liposome that survives dehydration. Further, as Dr. Daftary notes in his Declaration, it is not predictable how different buffers (i.e. Janoff's sugar, sodium chloride and HEPES versus Hong's ammonium sulfate) interact with different liposome components (i.e. Janoff's egg lecithin versus Hong's DSPC and Cholesterol), especially when the process in which these solutions and liposomes are different (Janoff's dehydration versus Hong's lack of dehydration). See Declaration at para. 40. Thus, one can not simply consider the volume of hydration media irrespective of difference in the constituents of the hydration media and the process as well as the liposome components. See id. A liposome is formed when phospholipid is hydrated, that is when thin film of phospholipid is contacted with water. But the bilayer membrane formed is modified in structure with certain substances included in the hydration

medium. To get a particular structure giving a specific performance of liposome other things being equal, hydration medium is critical. Use of NaCl and HEPES buffer along with sucrose in Janoff is critical, and to say that NaCl-HEPES is not critical and only sucrose is critical without any experimental evidence is not persuasive as it is contrary not only to the understanding of one of ordinary skill in the art, but also of one skilled in the art of liposome preparation. See Declaration at para. 39.

"removing extra liposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution"

To try and overcome Hong's deficiency relating to "removing extra liposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution," the examiner brings in Papahadjopoulos and Barenholz. The Examiner says that "Papahadjopoulos teaches the routine practice of inclusion of histidine in the hydration buffer." (Emphasis added). Dr Daftary has clearly pointed out the flaw in the argument that the combination of Papahadjopoulos with Hong motivates one of ordinary skill in the art to use histidine-sucrose buffer for dialysis at para 41.

Papahadjopoulos' hydration step procedure is also different from Hong. Applicants point out that the present claims require a sucrose-histidine buffer in the dialysis and the claims do not require any histidine in the hydration buffer. The use of histidine by Papahadjopoulos in the hydration buffer is, therefore, irrelevant. A hydration buffer is used to hydrate and form the liposomes whereas the dialysis buffer is used to remove extra-liposomal salt. These clearly are two different solutions for two different purposes used at different times. Thus, there is no motivation to take histidine used in Papahadjopoulos for hydration of liposomes and use in with sucrose in a dialysis solution. See Declaration at para. 41. The claim language used in the present claims clearly indicate that there are two different and distinct solutions, each used for a different purpose: one is a hydration medium consisting essentially of ammonium sulfate and sucrose and the other is a sucrose-histidine buffer solution used in dialysis for removing extra liposomal ammonium sulphate.

Barenholz also does not teach or suggest another element of the claimed invention: "removing ammonium sulphate from extra liposomal hydration medium by dialysis using a

sucrose-histidine buffer solution." None of the Examples in Barenholz show any dialysis method or spell out any dialysis buffer. These details are not obvious from Barenholz, as nowhere in Barenholz this has been taught or suggested.

Barenholz discloses transmembrane loading of amphipathic therapeutic or diagnostic agents (active material) into liposomes using an ammonium transmembrane gradient. In the exemplified process, a lipid film is hydrated with aqueous ammonium sulphate containing desferal (desferoxamine mesylate). In each case, 5 ml of the solution was added to a film formed from 100 mg egg phosphatidyl choline. In contrast, the present claimed method requires an aqueous hydration media that consists essentially of ammonium sulfate and sucrose and the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present (which is not EPC). Barenholz thus does not teach or suggest the use of a hydration medium consisting essentially of ammonium sulphate and sucrose. Further, Barenholz's solution uses desferal, which the present invention does not (as the instant claim 1 recites "consists essentially of ammonium sulfate and sucrose").

II. Claims 1-8, 10, 12, 14-22 and 63-69 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Slater (US 6,355,268) in view of Janoff (US 4,880,635).

The Examiner combines Slater and Janoff and alleges that they render the claimed invention obvious. To do so, they must together teach or suggest each and every claim element. Slater and Janoff do not teach or suggest each and every claim element, even when combined. In any event, even combining these references, they do not teach or suggest each and every claim element. As such, applicants request withdrawal of this ground of rejection. Further, one skilled in the art would not be motivated to combine Janoff with Slater to arrive at the present invention. The deficiencies of the references and the lack of motivation to combine these references are discussed in more detail below.

A. Slater fails to teach or suggest all of the claimed elements, alone or in combination with Janoff (or any other cited art).

Slater fails to teach or suggest all of the claimed elements, alone or in combination with Janoff (or any other cited art). The Office Action makes one believe that inclusion of sucrose

and buffer in the hydrating medium, is the only missing element in Slater, but this does not correspond to facts. Slater lacks the amount of the hydration media used per mMole of phospholipids specified in Claim 1 of present invention. Also Slater uses pegylated phospholipids whereas the present invention is all about use of non-pegylated phospholipids. Further, Slater does not use sucrose-histidine buffer for removing extraliposomal ammonium sulphate as specified in Claim 1 of present invention. More particularly, Slater does not teach: 1) a nonpegylated long circulating liposome; 2) an aqueous hydration media consisting essentially of ammonium sulfate and sucrose; 3) the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present to form long circulating non-pegylated liposomes; and 4) removing ammonium sulphate from extra liposomal hydration medium by dialysis using a sucrose-histidine buffer solution. Hence, Slater cannot be considered as a close prior art.

One reading Slater would not be motivated to try and make a nonpegylated liposome using the method claimed in the present invention as Slater actually teaches away from the claimed method because Slater focuses on the use of a hydrophilic polymer (such as polyethylene glycol "PEG") in the manufacture of liposomes and reports that the preferred liposomes have PEG as its hydrophilic polymer. Thus, one skilled in the art would not read Slater and be motivated to make a liposome without the use of PEG. See Declaration at para. 42.

More particularly, Slater teaches that his liposomes are derivatized with a hydrophilic polymer. See Col. 2, lines 55-56. Hydrophilic polymers include polyethylene glycol. SeeCol. 3, lines 22-23. Preferred embodiments utilize polyethylene glycol. See Col 3, lines 24-26. Slater teaches that the hydrophilic polymers provide the long circulation time: "The outermost surface coating of hydrophilic polymer chains is effective to provide a liposome with a long blood circulation lifetime in vivo." See Col 7, lines 43-45. Accordingly, one looking to make a long circulating liposome without polyethylene glycol (a hydrophilic polymer) would not even look to Slater for any motivation or teaching regarding a nonpegylated long circulating liposome since Slater indicates that PEG is essential for a long circulating liposome. See Declaration at para. 43.

1. Slater does not teach an aqueous hydration media consisting essentially of ammonium sulfate and sucrose.

Slater does not teach or suggest an aqueous hydration media consisting essentially of ammonium sulfate and sucrose as required by the claims. Example 1 of Slater provides his preparation method. Slater uses an ethanol:lipid:ammonium sulfate hydration mixture. See Slater at Col. 20, lines 47-51. There is no teaching or suggestion of a hydration media consisting essentially of ammonium sulfate and sucrose. Another preparation method is shown in Example 4. The lipids were formed in this example by a solution of dextran-ammonium salt and ethanol. Again, there is no teaching or suggestion of a hydration media consisting essentially of ammonium sulfate and sucrose.

2. Slater does not teach the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present.

Slater does not also teach or suggest the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present to form long circulating non-pegylated liposomes as required by the claims.

3. Slater does not teach removing ammonium sulphate from extra liposomal hydration medium by dialysis using a sucrose-histidine buffer solution.

Slater does not also teach or suggest removing ammonium sulphate from extra liposomal hydration medium by dialysis using a sucrose-histidine buffer solution as required by the claims. In Slater, the ammonium sulphate and ethanol were removed from the external bulk aqueous phase immediately prior to remote loading of the active agent by hollow fiber tangential flow diafiltration. This step is clearly different than the claimed process. In the above step, the claimed process removes only the organic solvent, whereas the Slater process removes both ammonium sulphate and ethanol together by tangential flow diafiltration. In example 1 there is a step of removing extra liposomal ammonium sulphate along with ethanol using 10% sucrose solution. In example 4 there is a step involving removal of unentrapped dextran sulphate and ethanol with 8 volume exchanges using 350mM sodium chloride solution followed by 8 volume exchanges using a 10% sucrose solution. Thus, Slater does not teach or suggest the claim element of "removing ammonium sulphate from extra liposomal hydration medium by dialysis using a sucrose-histidine buffer solution." Just because Slater uses sucrose-histidine buffer in

final liposomal preparation, it cannot said to be obvious to use it in the dialysis step, as the examiner has argued. Slater himself does not use it that way. See Declaration at para. 45.

B. Janoff fails to teach or suggest all of the claimed elements, alone or in combination with Slater (or any other cited art).

The deficiencies of Janoff were discussed above. Applicants reiterate that Janoff does not teach "an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose." Nor does Janoff even relate to a method of liposome manufacture that results in a non-pegylated long circulating liposome. Instead, Janoff's hydration buffer was NaCl, HEPES and trehalose or other sugars. Further, Janoff does not teach the removal of extraliposomal salt with a sucrose-histidine buffer, but rather Janoff used a sodium buffer to get rid of the potassium buffer. Col. 11 lines 1-4. Finally as discussed above, Janoff does not teach or suggest the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present to form long circulating non-pegylated liposomes as required by the claims. Applicants note that that Janoff is using egg phosphatidyl choline and not the claimed phospholipids (DSPC or HSPC) so Janoff does not teach the claimed element of "the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present" as the present claims define phospholipid as either DSCPC, HSPC or mixtures thereof. Janoff thus fails to teach this element of the claim.

C. No motivation to combine Slater with Janoff.

Notwithstanding the deficiencies in Slater and Janoff, one skilled in the art would not be motivated to combine Slater with Janoff. Janoff relates to the use of sugar to protect liposomes during dehydration, whereas Slater is not concerned with dehydration but rather is concerned with retaining the labile topoisomerase inhibitor without opening a lactone by preventing its contact with aqueous phase inside the liposome. Slater uses pegylated phospholipid and makes pegylated liposomes to keep the labile therapeutic agent (a topoisomerase inhibitor) intact. Dr. Daftary has clearly pointed out in para 46 that the condition of liposome formation in Slater requires distribution of polymer on both sides. One skilled in the art would not consider removing the pegylation used by Slater and instead use the sugars in Janoff when the processes

taught by these references are completely different. See Declaration at para. 45-46. A summary of the differences are as follows:

i) Janoff has demonstrated that a sugar works to protect liposomes prepared from (pure 99 %) egg phosphatidyl choline during dehydration and used no other phospholipid. There is no reason for one of ordinary skill in art to believe that it will work for liposomes prepared from other phospholipids and sterols, as used in Slater. Further, Janoff did not show or suggest the use of sugar to offer any protection or any other property other than protection during dehydration of liposomes. Conversely, Slater does not consider dehydration nor does he mention it.

(ii) Further, the hydration medium used in Slater is ammonium sulphate or dextran ammonium sulphate or heparin sulphate (See Slater at col. 11, lines 5-20). After the liposomes are formed, the external medium is exchanged for one lacking ammonium ions: ammonium sulphate is replaced by NaCl or a sugar. In contrast, Janoff has sodium chloride and HEPES in the hydration medium along with sugar. Thus, Slater has and needs ammonium ions inside the liposomes where Janoff requires sodium chloride in the liposomes. Janoff has not shown that by using sucrose and water as hydration medium he has obtained liposomes that are stable to dehydration and rehydration. Dr Daftary has said in para 39 that NaCl and HEPES buffer in Janoff's hydration medium is critical in giving the performance of the liposome and it is not the sugar alone is the cause for this performance.

(iii) Janoff's process involves first removal of solvent from the lipids and then adding hydrating medium. In contrast, Slater's process first adds a hydration medium and then removes the solvent. Thus, Slater's process steps are in the reverse order to that of Janoff.

(iv) Slater does not dehydrate the formed liposomes. In contrast, Janoff dehydrates the formed liposomes.

In summary, there is no motivation to combine Slater and Janoff and further, even the combination of these references does not teach or suggest each and every claim element. As such, applicants request withdrawal of this ground of rejection.

III. Claims 1-8, 10, 12, 14-22 and 63-69 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Forssen (US 5,714, 163) in combination with Janoff (US 4,880,635)

and Papahadjopoulos (US 4,235,871), optionally in further combination with Slater (US 6,355,268) and/or Clerc (US 5,939,096).

The Examiner combines Forsse with Janoff and Papahadjopoulos (and optionally with Slater and/or Clerc) and alleges that they render the claimed invention obvious. To do so, they must together teach or suggest each and every claim element. These references do not teach or suggest each and every claim element, even when combined. Further, one skilled in the art would not be motivated to combine these references to arrive at the present invention. The deficiencies of the references and the lack of motivation to combine these references are discussed in more detail below.

A. Forssen fails to teach or suggest all of the claimed elements, alone or in combination with Slater (or any other cited art).

Forssen does not teach or suggest at least three elements of the claims: 1) an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose; 2) the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present; and 3) removing extra liposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution. A detailed analysis follows.

1. Forssen does not teach or suggest the claimed hydration medium.

Forssen relates to phosphatidyl choline/cholesterol (PC/CHOL) liposomes containing vincristine or other cationic vinca alkaloids and an anion in an aqueous phase of liposome. Reference is made to several prior art methods for forming liposomes and there is no suggestion that Forssen teaches any new or modified method of liposome formation. It is stated that a significant benefit of the liposomes used by Forssen is that they can be prepared without a transmembrane or pH gradient (see column 4, lines 26-30). In the generally exemplified process, PC/CHOL is hydrated with aqueous anion and the vinca alkaloid is loaded by ion-exchange loading (see column 4, line 49 to column 5, line 10).

In Example 1 of Forssen, a spray-dried distearoylphosphatidylcholine/cholesterol (DSPC/CHOL) lipid was hydrated with either a buffer containing the ammonium salt of one of the counter-ions or a buffer containing 300 mM sucrose. The Example goes on to note that

actually 10 different buffers were used – all were 10 different salts of ammonium. Of the 10 buffers used, one contained ammonium sulfate. After sonication, annealing, centrifuging and buffer exchange by gel filtration on a Sephadex column previously equilibrated with unbuffered 300 mM sucrose, vincristine was ion-exchange loaded by incubation of the liposomes with an aqueous solution of its sulfate salt.

Applicants direct the Examiner to Table 1 of Example 1 of Forssen (col. 6, line 53 to col. 7 line - 14). When the counter ion is tartrate, the percent of vincristin entrapped is 90% and when the counter ion is sulphate, the percent of vincristin entrapped drops to 49%. This table shows that different counter-ions have different entrapment rates. The Examiner has not explained why one skilled in the art looking at Example 1 of Forssen would choose sulphate as a counter ion especially when there are other ions (except succinate) giving entrapment above 67%. If one reads the same example completely, further down in the animal experiment there is a sentence in column 7, lines 26-32 that reads as follows:

> The mice were randomized into 11 treatment groups and therapy was initiated four days after tumor implantation. The chemotherapeutic treatment groups consisted of free Vcr and nine vesicle-Vcr formulations (Vcr salts of glutamate, tartrate, hydrogen diphosphate, aspartate, EDTA, succinate, pyrophosphate, lactobionate, and citrate). Dosing was at 2.5 mg/kg.

Notably from this passage, sulphate is not at all used, which clearly shows that the inventors of Forssen did not think ammonium sulphate was all that useful based on its low entrapment.

Thus, even though there is mention of an ammonium sulphate for drug loading, there is no teaching or suggestion to use ammonium sulphate in a solution with sucrose to hydrate/form liposomes as required by the claims. Further, one would not even be motivated to use ammonium sulfate in other drug loading procedures because of its poor performance, let alone be motivated to combine with sucrose and use in a different step altogether – liposome hydration. See Declaration at para. 47.

Forssen also shows that changing one small component in the manufacture of liposomes can alter their behavior dramatically. Changing one counter ion from tartarte to sulphate causes the entrapment procedure to drop in efficiency by over 50%. Thus, this shows, contrary to the Examiner's belief, that one can not simply interchange solutions, process steps, and liposome

components and have any sort of predictable outcome. See also Declaration of Dr. Daftary at para. 27.

Thus, there is no teaching in Forssen that would have led one of ordinary skill in the art to conclude that the presence of both ammonium sulfate and sucrose in an aqueous medium for hydrating phospholipid would reduce leakage from a loaded liposome. Moreover, there is no reason to conclude from Forssen that sucrose would be required for prolonging circulation. Thus, Forssen does not teach or suggest the claimed element and nor would there be any motivation to combine with the other cited prior art.

2. Forssen does not teach or suggest the use of 10 to 35 ml for each mmole of phospholipid present.

Forssen also does not teach or suggest the use of 10 to 35 ml for each mmole of phospholipid present. There is no mention of how much hydration buffer is used to form the liposomes.

3. Forssen does not teach or suggest the claimed sucrose histidine buffer.

Moreover, Forssen does not use sucrose-histidine buffer for removing extra liposomal hydration salt. This feature is not an obvious process step, especially in view of the deficient art, and by itself has inventive merit, in achieving removal of hydration media salts and depositing sucrose on the outside layer of the liposome after the liposomes are sized. See Declaration at para. 47.

These deficiencies in Forssen are not cured by the cited references of Janoff, Papahadjopoulos, Slater, and/or Clerc. As discussed above, Janoff, Papahadjopoulos, and Slater also do not teach or suggest, alone or in combination all of the recited claim elements. More particularly, they do not also teach: 1) the use of a hydration medium consisting essentially of ammonium sulfate and sucrose; 2) the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present and; 3) a sucrose-histidine buffer for removing extra liposomal hydration salt. Further, as noted below, Clerc also fails to teach or suggest all of the claimed elements, alone or in combination with Forssen/Slater (or any other cited art).

B. Clerc fails to teach or suggest all of the claimed elements, alone or in combination with Forssen/Slater (or any other cited art).

1. Clerc does not teach or suggest the claimed hydration medium.

Clerc does not teach or suggest an aqueous hydration media consisting essentially of ammonium sulfate and sucrose. In describing the hydration medium, Clerc states that the hydration medium is preferably at least 50 mM weak acid salt, which is described in the specification as: a sodium/calcium acetate, sodium/calcium formate, salts of propanoic, butanoic or pentanoic acids. See Col. 7, lines 15-20. There is thus no teaching of a hydration medium consisting essentially of ammonium sulfate and sucrose.

2. Clerc does not teach or suggest the use of 10 to 35 ml for each mmole of phospholipid present.

Clerc does not teach or suggest an aqueous hydration media consisting essentially of ammonium sulfate and sucrose and also does not teach that the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present to form long circulating non-pegylated liposomes as required by the claims.

3. Clerc does not teach or suggest the claimed sucrose histidine buffer.

The Office Action states "while disclosing a therapeutic or diagnostic agent (active material) loading method into the liposomes teaches the hydration of the phospholipids with a solute species which is saline or a disaccharide (sucrose) and a buffer which is the same as the internal or external aqueous medium such as histidine or MES or Tris" (Col.7, lines 5-15; col. 8 lines 8-15). Notably, when referring to the solute species, Clerc is describing external mediums that are used to produce a higher inside/lower outside concentration of the weak acid salt, and is not referring to a hydration medium. Clerc states:

> The external medium preferably contains (i) a buffer, e.g., a 5-50 mM buffer having a pH the same as or similar to the pH of the original hydration medium, and (ii) solute species effective to raise the osmolality of the external medium to close to that of the internal medium, e.g., 200-300 osm/kg. The buffer

> is preferably one, like histidine, MES or TRIS, which is relatively impermeant, and which exerts maximum buffering capacity in the pH 5-7 range.
>
> The solute species for external-medium osmolality is preferably either the salt of a strong acid, e.g., physiological saline, or a mono- or di-saccharide, such as sucrose, glucose, or mannitol. The latter type of solute is preferred where it is desired to store the liposomes by lyophilization, in which case the saccharide functions as a cryoprotectant to minimize liposome damage during freezing and rehydration.

See Col. 8, lines 1-16. Clerc is thus describing a solution used for external-medium osmolality and is not referring a buffer used to remove ammonium sulphate from the extra liposomal hydration medium as required by the claims. Thus, Clerc does not teach or suggest the element of claim 1 of "removing ammonium sulphate from extra liposomal hydration medium by dialysis using a sucrose-histidine buffer solution" as the buffers used in Clerc are instead used to form a liposome pH gradient.

There is no motivation to combine Clerc with Forssen/Slater. Clerc is describing his method as a method of forming liposomes having a higher inside/lower outside pH gradient. The weak acid compounds for loading by his method include ibuprofen, tolmetin, indomethacin, phenylbutazone, meclofenamic acid, piroxycam, ciprofloxacin and nalidixic acid. There is no reason to look at it for increasing circulating time of liposome without a PEG coating or providing a graft of a hydrophilic polymer chain as mentioned by Clerc. It would not be obvious to one of ordinary skill in the art, to take only from that portion indicated by examiner in Clerc for making the instant invention when the instant invention does not follow a higher outside/lower inside pH gradient system. The instant invention also does not follow providing a coating or a graft of hydrophilic polymer chain to increase the circulating time of liposomes. There is no motivation to take any teaching from Clerc, and no motivation to choose a particular internal/external medium from alternatives suggested in the portion indicated by the Examiner. (See Declaration at para. 48).

Forssen, Janoff and Papahadjopoulos do not have any teaching on circulation time of the liposome. Therefore there would not be any motivation to make a combination of these three documents for making a liposome as per this combination for getting a long circulating liposome. Clerc also has no reference to circulation period of liposome. Slater does reference circulation time, but achieves this with a pegylated liposome (unlike the present invention). It was clearly known that pegylation increases circulation time so there is no motivation to

combine any step from Slater process when pegylated phospholipids are not part of the present invention.

For a bona fide obviousness rejection, the proffered combination of references must hint at some level of expectation of success by one skilled in the art. In the present case, it is clear that one skilled in the art would not have combined these references because they would not have expected to achieve a long circulating non-pegylated liposome. The combination of various elements from the cited references would have been undertaken by one skilled in the art (as clearly set forth in Dr. Daftary's Declaration) because the combination proffered by the Examiner ignored the references and how they used the certain elements. Further, even taking the erroneous combination of references, they still do not teach or suggest each and every element of the claims. Accordingly, applicants request withdrawal of the grounds of rejection. A claim chart is provided below summarizing the claim elements missing in the cited references.

Secondary Considerations of Nonobviousness

Dr. Daftary has indicated in his Declaration at para. 5 and 50 that the instant invention process is enabling and has been successfully commercialized. Not until the present inventors invented the claimed process (which was 13 years after Janoff and 22 years after Papahadjopoulos) did someone think of adding sucrose to the hydration medium, and/or maintain volume of hydration medium and/or to use histidine sucrose medium to remove extraliposomal ammonium sulphate in dialysis medium, as in instant claim 1, to get a liposome with increased circulation time without the use of PEG.

Appendix 1: Claim Chart

10/748,094 (instant application)	Aqueous hydration media consisting essentially of ammonium sulfate and sucrose	10-35 ml for each mmole of phospholipid (DSCPC/HSPC or mixtures thereof)	Removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution
Hong	No No mention of sucrose in a hydration medium	No	No
Forssen	No Buffer with EITHER ammonium salt of one of the counter-ions OR 300mM sucrose but no mention of hydration buffer that uses both	No	No
Janoff	No Mentions sugars in the bilayer for the purpose of protective the internal contents during dehydration steps not for hydration. No mention of a hydration medium consisting essentially of ammonium sulfate and sucrose.	No Does not have the phospholipids required by the claims.	No
Papahadjopoulos	No	No	No Mentions histidine buffer in hydration buffer step. No mention of a histidine sucrose solution for removal of extraliposomal ammonium sulphate salt
Slater	No	No	No
Clerc	No	No	No

TABLE 1: Hydration step and other differentiating details

Reference	Lipid = Phospholipid, sterol etc.	Hydration medium	Volume (ml/mM of Phospholipid) (Rough calculations)	Sucrose-histidine buffer in dialysis*	Other details
Hong	DSPC:Cholesterol - 3:2 + PEG-DSPE	Ammonium sulphate 250 mM pH 5.0	No data	No Step	Particle size is between 65 to 75 nm. 1mg Doxorubicin loading per 10μM of phospholipid
Papahadjo-poulos	Phosphatidyl-glycerol 10 μM + phosphatidyl-choline 40 μM + cholesterol 50 μM	NaCl 10 mM + 4mM histidine / TES	3ml (1.5ml + 1.5ml) 60 ml/mM of Phospholipids	No Step	Oligolamellar vesicles, particle size between 0.2 to 0.6μ. Percentage of encapsulation is 34%. Bio-gel A 1.5 agarose column to separate unencapsulated material.
Janoff	EPC 80 μM	150 mM NaCl + 20mM HEPES + sugar –Aq soln.	2 ml (25ml/mM)	No Step	Unencapsulated material removed by Sephadex G-50 or Ultragel AcA 34 column. Dehydration of liposomes.
Barenholz	100 mg (= 0.132 mM) egg phosphatidyl-choline (mol. wt. 760)	Aqueous ammonium sulphate containing desferal	5ml/0.132mM 38 ml/mM	No Step	
Slater	HSPC + Cholesterol + PEG-DSPE (56.4 :38.3::5.3) Mol.	250mM ammonium sulfate solution; Dextran sulphate ammonium salt solution	3.7g total lipid in 100ml ammonium sulphate solution. 32.6ml/mM of phospholipid	Diafiltration Ammonium sulfate is replaced by sodium chloride or Sucrose	$T_{1/2}$ of drug entrapped in pegylated liposome 32 – 36 times that of free drug
Forssen	DSPC + Cholesterol 2:1M 100 mg lipid /ml on hydration	Ammonium salt (tartarate, sulphate etc.) solution	80.3 mg DSPC/ml (9.8ml/mM)	Buffer exchange on Sephadex col.	
Forssen	DSPC + Cholesterol 2:1M 100 mg lipid /ml on hydration	Sucrose 300mM no results		Buffer exchange on Sephadex col.	
Clerc	HSPC: cholesterol – 60:40 M	150 mM weak acid salt – Na acetate/ 120mM Ca acetate acetate/formate; salts of propanoic, butanoic or	Vol. adjusted to obtain 10 %w/v lipid conc. i.e. 6g HSPC/100ml, 13.17 ml/mM of Phospholipid	No Step	

		pentanoic acids			
Present invention	DSPC and/ or HSPC 1mM	Ammonium sulphate and sucrose	10-35 ml per mM of phospholipid present	Removing ammonium sulphate extra liposomal hydration medium	$T_{1/2}$ of drug entrapped in non-pegylated liposome of Example II is 49 times that of free drug

*For removal of ammonium sulphate from extraliposomal hydration medium

TABLE II: Combination of references

Combination 1: Hong, Papahadjopoulos, Janoff and optionally Barenholz

Two combined	Combined for	Reason why combination is not persuasive
Hong + Papahadjopoulos	1. Volume of hydration medium	1. a) The process of hydration is different from that in Hong, and it is not compatible with that in Hong. Papahadjopoulos process is a two stage process, it goes through a gel stage, So also hydration in presence of solvent is not compatible with process of Hong,(Hong example 2). b) volume of the hydration media is outside to that in instant claim 1, which is for mMole of phospholipid and not per total lipid. This volume in both examples 1 and 2 is not 1.5 ml, it is 1.5 ml twice one before gel formation and another after gel formation when liposome is formed. (See Page 19). c) hydration medium and phospholipids used in the two processes are different and are different from instant claim 1 (Lipids contain phosphatidylglycerol). d) Objective of Papahadjopoulos is to increase circulation time using lipid polymer conjugate. (See page 15 and Declaration para. 35, 36)
	2. Inclusion of histidine in hydration buffer	2. This is not relevant, instant claim 1 does not use histidine in hydration buffer (See page 23 and Declaration para. 41).
Hong + Janoff	1. Sucrose inside and outside liposome retains Adriyamycin during dehydration and rehydration	1. a) There is no knowledge before this application that presence of sucrose would increase circulation period. (See pages 16 – 18 , Declaration para. 29). b) In Janoff sucrose with HEPES and NaCl is inside liposome and not just sucrose. Separating sucrose from HEPES and NaCl and combining it with ammonium sulphate in Hong is not obvious. (See Page 16 – 18 Declaration para. 32). c) Janoff teaches dehydration and rehydration of liposomes and not liposomes with longer circulation time. (See page 16 – 18, Declaration para. 29). d) Janoff's stability is tested of dehydrated liposomes (moisture less than 2%) stored in refrigerator up to 7 days and there is no indication whether it will stand in aqueous conditions while circulating in blood at body temperature. (See page 21, TABLE I and Declaration para. 28).

	2. Volume of hydration media	1. Lipid (EPC) used in Janoff is different, hydration medium used is different from Hong and instant claim 1, volume of hydration medium, being dependent on these factors, is not relevant for applying to Hong hydration medium with any expectation of success in raising circulation period. (See page 20, 21; Declaration para-37).
Hong + Barenholz	Removal of outside ammonium sulphate	Barenholz, does not have the step of removal of extraliposomal ammonium sulphate by dialysis using sucrose-histidine buffer, and it cannot make this step obvious. a) Neither Hong nor Barenholz teach removal of ammonium sulphate by dialysis using sucrose histidine buffer as taught in instant claim 1. And there is no evidence to believe that that such a step would contribute, along with other features of the instant invention, to raising circulation rate of the liposome formed, without the gleanings from the present claim 1. (See page 15,16 and Declaration para 33). b) In the present Invention, it is not removing just ammonium sulphate from the extraliposomal media but while doing so it also improves in parts the desired structure to the liposome with right permeability of the liposomal membranes to give longer circulation period and deliver the drug slowly over the extended period of circulation. (See TABLE I and Declaration para 28.)

Combination 2: Slater and Janoff

Two references combined	Combined for	Reason why combination is not persuasive
Slater + Janoff	1. Having sucrose in hydration buffer	1. As in Hong and Janoff in Combination 1 above.
	2. hydration volume	2. Lipid (EPC) used in Janoff is different, hydration medium used is different from Slater and instant claim 1, volume of hydration medium being dependent on these factors is not relevant for applying to Slater hydration medium with any expectation of success in raising circulation period. (See page 31, TABLE I and Declaration para 23).

Combination 3: Forssen, Janoff, Papahadjopoulos, optionally Slater and/or Clerc.

Two references combined	Combined for	Reason why combination is not persuasive
Forssen + Janoff	1. Having sucrose in Hydration medium:	1. As in Hong and Janoff in Combination 1 above.
	2. Hydration volume	2. Lipid (EPC) used in Janoff is different, hydration medium used is different from Forssen and instant claim 1, hence volume of hydration medium, being dependent on these factors, is not relevant for applying to Forssen hydration medium with any expectation of success in raising circulation period. (See page no. TABLE I and Declaration para 23).
Forssen + Papahadjopoulos	1. Volume of hydration medium	1. as in Hong and Papahadjopoulos in Combination 1 above.

	2. Inclusion of histidine in hydration buffer	2. This is not relevant, instant claim 1 does not use histidine in hydration buffer (See TABLE I and Declaration para. 41).
Forssen + Slater	Removal of outside ammonium sulphate	Slater does not make the step of removal of extraliposomal ammonium sulphate by dialysis using sucrose-histidine buffer, obvious, because Slater does not suggest this element. a) Forssen does not teach use of sucrose histidine buffer for removal of ammonium sulphate. (See TABLE I and Declaration para. 44)
Forssen + Slater +/or Clerc	Composition of hydration buffer	Forssen + Slater as alternative to show obviousness is not persuasive as said above. Clerc does not teach hydration medium composition as in Claim 1 of the present invention. (See TABLE I)

CONCLUSION

In view of the remarks above, Applicants respectfully submit that this application is in condition for allowance and request favorable action thereon. The Examiner is invited to contact the undersigned if any additional information is required.

Applicants authorize the Commissioner to charge Deposit Account No. 042223, referencing Attorney Docket No. 067080-0003 for any fees due for filing this paper and to credit any overpayments.

Respectfully submitted,

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